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Organic loading rate impact on biohydrogen production and microbial communities at anaerobic fluidized thermophilic bed reactors treating sugarcane stillage

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HIGHLIGHTS

- Thermophilic H_2 production at high OLR from sugarcane stillage was feasible.
- The volumetric H_2 production and OLR demonstrated a positive correlation.
- H_2 yield at both AFBR reactors initially increased by a threshold OLR value.
- An optimum OLR for maximum H_2 yield was 60.0 kg COD m^{-3} d^{-1}, at 15,000 mg COD L^{-1}.
- Hydrogen producer *Megasphaera* sp. and *Lactobacillus* sp. were identified.

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ABSTRACT

This study aimed to evaluate the effect of high organic loading rates (OLR) (60.0–480.00 kg COD m^{-3} d^{-1}) on biohydrogen production at 55 °C, from sugarcane stillage for 15,000 and 20,000 mg COD L^{-1}, in two anaerobic fluidized bed reactors ($AFBR_1$ and $AFBR_2$). It was obtained, for H_2 yield and content, a decreasing trend by increasing the OLR. The maximum H_2 yield was observed in $AFBR_1$ (2.23 mmol g COD_{added}^{-1}). The volumetric H_2 production was proportionally related to the applied hydraulic retention time (HRT) of 6, 4, 2 and 1 h and verified in $AFBR_1$ the highest value (1.49 L H_2 h^{-1} L^{-1}). Among the organic acids obtained, there was a predominance of lactic acid (7.5–22.5%) and butyric acid (9.4–23.8%). The microbial population was set with hydrogen-producing fermenters (*Megasphaera* sp.) and other organisms (*Lactobacillus* sp.).

1. Introduction

Wastewaters with high concentrations of organic pollutants (Castro-Villalobos et al., 2012) are increasingly being produced worldwide. Anaerobic digestion of these compounds is an attractive alternative, since renewable energy obtained from biomass and organic compounds can provide continuous production of energy source in the form of biogas. Accordingly, due to its inexhaustible potential, low cost, and because it is considered a renewable source of clean energy, the use of biohydrogen has attracted global attention (Show et al., 2012). The bioproduction of H_2 from wastewater is a sustainable alternative to an economy based on fossil fuels, once this biogas is a high yield energy carrier.

Studies are being directed to investigate the fermentative production of hydrogen using real wastewater with high organic load rate at continuous reactors, for example, from coffee drink manufacturing (Jung et al., 2010), rice straw waste (Tawfik and Salem, 2012), molasses (Han et al., 2012) and palm oil (Singh et al., 2013).

Countries with agribusiness economies have potential for significant economic development through the incorporation of bioenergy industry (Show et al., 2012). In this sense, it can be noted that ethanol industry produces large amounts of stillage, considered the main effluent generated from the sugarcane processing. The elevated amount generated, around 8–18 $L_{stillage}$ $L_{ethanol}^{-1}$, the high organic content, the presence of potassium, calcium, magnesium, phenolic compounds and melanoidins responsible for the brownish color, and low pH, are features that make this effluent a recalcitrant pollutant with high environmental impact (Ferreira et al., 2011), and thus, subject to anaerobic treatment. The high

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temperature of the effluent release, around 90 °C, makes this wastewater even more viable for microbial conversion to hydrogen, ensuring the thermophilic fermentative production.

Hydrogen production from real wastewater, among other factors, is strongly influenced by the organic loading rate (OLR) applied in the reactors. The OLR depends on the hydraulic retention time (HRT) and on the organic matter concentration of the substrate supply. Therefore, it is essential to define a range of OLR, which will be possible to achieve constant efficiency in the biological reactor, or an optimum OLR value for maximum H_2 yield. As a result, the fermentative routes and final metabolites products may be modified due to the OLR applied, as well as the conversion efficiency of the substrate and the microbial community established in the system (Gioannis et al., 2013). Some studies reported that high OLR might decrease hydrogen yield (HY), while in others, high values of OLR could raise HY (Tawfik and Salem, 2012). Han et al. (2012) used molasses as wastewater, with concentrations from 2000 to 8000 mg COD L^{-1}. The authors stated that from the OLR among 8 to 32 kg COD m^{-3} d^{-1}, the H_2 production at a CSTR reactor initially increased until the threshold of 24 kg COD m^{-3} d^{-1}, with a consequent trend of decreasing hydrogen production at OLR of 32 kg COD m^{-3} d^{-1}. Using palm oil as substrate, Singh et al. (2013) modified HRT in the range of 24–6 h (OLR from 38.4 to 158.4 kg COD m^{-3} d^{-1}), reaching lower hydrogen production and yield at elevated HRT.

The aim of this study was to evaluate the effect of high OLR applied, obtained from sugarcane stillage, with concentrations of 15,000 and 20,000 mg COD L^{-1} on biohydrogen production, encompassing the fermentation process, organic acids distribution, and monitoring the microbial community along the operational phases. Hydrogen production and yield were evaluated, as well as the prevalence and distribution of organic acids compounds, substrate consumption, and the relations of nitrogen, phosphate and sulfate in different organic loading rates applied at two anaerobic thermophilic fluidized bed reactors ($AFBR_1$ and $AFBR_2$). Elevated OLRs were investigated, among 60.0 and 480.0 kg COD m^{-3} d^{-1}, varying the HRT from 6, 4, 2 and 1 h.

2. Methods

2.1. H_2 production inoculum

The thermophilic AFBR reactors were inoculated with a granular sludge of a thermophilic upflow anaerobic sludge blanket reactor (UASB), used for the treatment of stillage from sugarcane, for biogas production (CH_4), located at the São Martinho distillery plant (Pradópolis, SP, Brazil), a producer of sugar and ethanol from sugarcane as raw material. To inhibit methanogenesis in thermophilic AFBR reactors, it was conducted heat pre-treatment of the inoculum, as performed by Shida et al. (2012).

2.2. Wastewater: sugarcane stillage

The organic substrate, sugarcane stillage, used as feeding wastewater to the AFBR thermophilic reactors for hydrogen production was collected at the São Martinho distillery plant (Pradópolis, SP, Brazil). Nutrients necessary for cell growth were added in the influent of the reactors, according to Shida et al. (2012). There was no addition of acidifying or alkalizing agents in the influent of the reactor.

The characteristics of the raw stillage were evaluated considering the physical–chemical aspects, such as pH, total organic carbon (TOC), total COD, total nitrogen, total phosphorus (as PO_4^{3-}), total sulfur (as SO_4^{2-}), zinc, manganese, copper, calcium, magnesium, potassium, the composition and distribution of organic acids (Table 1).

Around 30,000 mg COD L^{-1} of the raw stillage was diluted to obtain 15,000 mg COD L^{-1} at the $AFBR_1$ and to obtain 20,000 mg COD L^{-1} at the $AFBR_2$. The lowest and the highest COD:N:P ratios achieved from the wastewater during the OLR increase were 170:6:1 and 195:5:1, respectively, at the $AFBR_1$. Concerning the $AFBR_2$, the ratios were 215:9:1 and 237:9:1. The COD:SO_4^{2-} ratio, in both reactors, was among 13:1–19:1.

2.3. Thermophilic AFBR reactors ($AFBR_1$ and $AFBR_2$)

The reactors were constructed from transparent acrylic with the following dimensions: a thickness of 5 mm, height of 120 cm, internal diameter of 5.3 cm and a volumetric capacity of 2646 cm^3.

The characteristics of the expanded clay used as the support material for biomass immobilization and adhesion were as follows: grain sizes between 2.8 and 3.5 mm, real density of 1.5 g cm^{-3} and porosity of 23%. Approximately 800 g of expanded clay was introduced into the reactor, providing a static bed 40 cm tall in the AFBR reactors. The placement of a U-shaped tube in the bed of the reactors and the use of a thermostatic jacket, inside of which circulated water from a thermostatic bath at 65 °C, operating alone and/or simultaneously, maintained a uniform thermophilic temperature of 55 ± 1 °C.

2.4. Startup procedure and thermophilic reactors operation

As described above, the high organic content of the raw wastewater was diluted with water for use in the feeding solution

Table 1
Physical–chemical characterization of the raw sugarcane stillage (non-diluted).

Values	Parameters															
	pH	TOC[c] (ppm)	COD_{total}[d]	N_{total}[e]	P_{total}[f]	SO_4^{2-}	Zn	Mn	Ca	Mg	K	HLa[g]	HAc[h]	HBu[i]	HIsBu[j]	HPr[k]
			mg L^{-1}													
Min[a]	4.87	4598	30,406	700	180	1800	0.97	3.70	698	367	3800	5960	526	497	2367	582
Max[b]	5.06	5235	33,015	1200	240	2600	2.14	5.00	757	580	4500	7889	1617	613	4597	874

[a] Min: minimum.
[b] Max: maximum.
[c] TOC: total organic carbon.
[d] COD: chemical oxygen demand.
[e] N total Kjedahl.
[f] P total as PO_4^{3-}.
[g] HLa: lactic acid.
[h] HAc: acetic acid.
[i] HBu: butyric acid.
[j] HIsBu: iso-butyric acid.
[k] HPr: propionic acid.